

Potential Effect of Modified Chitosan in Environmental Treatment: Metal Uptake, Ion Selectivity and Bioactivity

ABSTRACT

Chitosan (CS) reacted with different organic reagent to give CS-CA, CS-CN, and CS-TEOF. The CS-TEOF reacted with 2-aminothiophenol, 2-aminophenol and phenyl hydrazine to give the CSTEOP-ATP, CSTEOP-AP and SCTEOF-PH, respectively. The modified chitosan derivatives were representing the N-S, N-O and N-N, as chelating centers were treated with the aqueous solution containing a mixture of different metal ions (Cu^{2+} , Cd^{2+} , Fe^{3+} , Co^{2+} , Pb^{2+} , Mn^{2+} , Hg^{2+} , Cr^{3+} , Pd^{2+}) to investigate their selectivity and their metal uptake capacity. The N-O chelating center selective Fe, Co and Cu while O-O selective for Pb, Cr, Ca, Ni, Cd and Pd. The N-S was selective to Cu(II) than N-N chelating center. The removal capacity by modified chitosan ligands for Ca^{2+} , Cd^{2+} , Fe^{3+} , Co^{2+} , Pb^{2+} , Mn^{2+} exceed 80%. The morphology of the modified polymer and their metal complexes were studied. The bioactivity of chitosan was improved through modification with sulfur containing compounds.

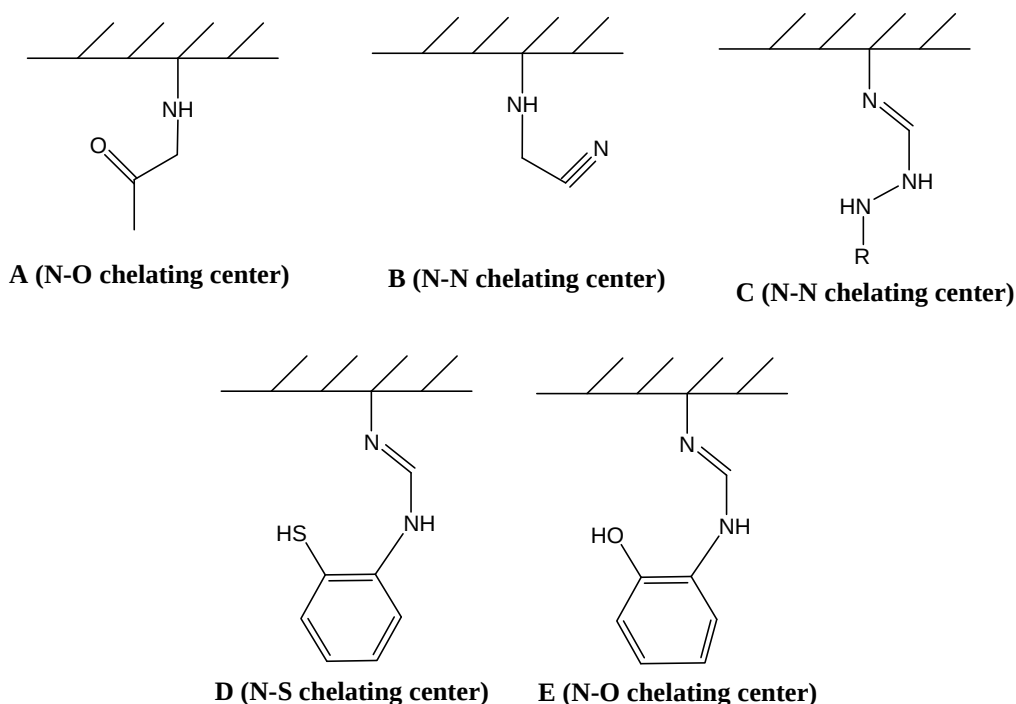
Keywords: Chitosan, wastewater treatment, metal complex, bioactivity

1. INTRODUCTION

Chitosan modifications have received great attention in last few decades. Polyfunctional chitosan determines one of the highest among natural polysaccharides metal binding ability. This enables chitosan application as a ligand not only for recovery, concentration of metal ions and separation, but as a matrix component of metal-containing catalysts, optical devices, chromatographic substrates, medicinal drugs and antimicrobial [1]. The potential effects of chitosan in the environmental treatment for metal removal of different fields have attracted great interest [2-13]. Industry considered as one of the major sources of heavy metal pollution of water [14-16].

The good behavior of chitosan toward metal binding have promoted a steady growth of interest in theoretical and experimental studies and its derivatives interactions with metal ions [17–19] and the growth of simple and economically methods for the preparation of derivatives with increased selectivity and sorption [2,3,20–23]. Heavy metals are accumulating in living organisms causing many diseases such as cardiovascular effects, bladder, skin lesions, lung cancers, and anemia [24]. Ions of elements such as Hg(II), Pb(II),

32 Cr(III), Cd(II), Co(II), Cu(II) and Ni(II) are toxic to the aquatic systems. It is well-known
33 that the Cr (VI) is toxic to living systems and must be removed from wastewater before it can
34 be discharged. The removal of Cr (VI) through acid catalyzed chemical reduction of Cr^{3+} and
35 then precipitation as hydroxide at alkaline conditions was reported [25-27]. The Cu ions at
36 certain level of concentrations it can become toxic to humans, causing cancer and promoting
37 oxidation. The removal of Cu by chitosan and precipitation as copper hydroxide by lime was
38 also reported [28, 29]. On the other hand, chitosan and its derivatives have attracted
39 considerable interest due to their antimicrobial and antifungal activities [30]. The chitosan
40 metal complex showed an effect on microorganisms. Wang *et al.*[13] mentioned that after
41 chitosan binding the metal ions through oxygen, nitrogen or both, the bindings are likely to
42 leave some potential donor atoms free which enhance the bioactivity. Chitosan is used as a
43 pharmaceutical agent in biomedicine, a human dietary supplement for weight loss,
44 cholesterol reduction, a hydrating agent in cosmetics, and an antimicrobial food wrap [31-
45 34]. In advance the antimicrobial activity of chitosan showed that low molecular weight
46 chitosan exhibited strong bactericidal activities as compared by that with high molecular
47 weight. The result of antibacterial activity of chitosan–copper complexes against *Salmonella*
48 indicated that the complexes with molar ratio close to 1:1, exhibited inhibition rate reached
49 100% [6]. In this article chitosan was modified to give new types of chelating centers like N-
50 O (structure A & E), N-N (structure B & C), N-S (structure D), and O-O (oxygen atoms of
51 chitosan) which would increase the capacity and metal uptake selectivity (cf. Scheme 1). On
52 the other hand, we aimed to increase the bioactivity of chitosan by its modification.
53 Bioactivity of the modified chitosan and metal complex against bacteria and fungi were
54 investigated. The structures were established based on elemental analysis, spectral data, SEM
55 and XPS.



Scheme 1. Suggested and aimed chelating centers

2. Experimental

2.1. Materials

Chloroacetonitrile, chloroacetone, triethyl orthoformate (TEOF), 2-aminothiophenol and 2-aminophenol was used as received. All metal salts and other chemicals were reagent grade and used without further purification.

2.2. Characterization Methods

2.2.1. Elemental Analysis

Elemental analysis was performed at Analab, on LECO CHNS – 932, Faculty of Science, Kuwait University.

2.2.2. IR Spectra

IR spectra are recorded as KBr discs of monomers, polymers and the metallo-polymer complexes on Perkin-Elmer System 2000 FT-IR spectrophotometer (Kyoto, Japan).

2.2.3. Scanning Electron Microscopy (SEM)

72 Scanning Electron Microscopy (SEM) for the polymers and their metallopolymer complexes
73 were examined at room temperature in a model JSM 6300 JEOL scanning electron
74 microscope (Akishima, Japan) at 20 kV.

75 2.2.4. X-Ray Photoelectron Spectroscopy (XPS)

76 XPS spectra were recorded on a Thermo ESCALAB 250 Xi using monochromatic Al K α
77 radiation (1486.6eV) with a spot size of 850 μ m. The spectra acquisition and processing were
78 carried out using the software Thermo Advantage, version v5.956.

79 XPS analysis was done on a sample containing C, N, O and metal. XPS spectra were
80 recorded on a Thermo ESCALAB 250 Xi using monochromatic Al K α radiation (1486.6eV)
81 with a spot size of 850 μ m. The spectra acquisition and processing were carried out using the
82 software Thermo Advantage, version v5.956. The sample was stuck on the sample holder
83 using a double-sided carbon tape and then introduced into the preparation chamber and was
84 degassed until the proper vacuum was achieved. Then it was transferred into the analysis
85 chamber where the vacuum was 10⁻¹⁰ mbar. The analysis was carried out using the following
86 parameters: Pass energy of 20eV, dwell time 50ms and the Step size 0.1eV. The XPS spectra,
87 both the deconvoluted and non-deconvoluted are shown in the figures.

88 2.3. Synthesis

89 2.3.1. Preparation of CS-TEOF: Reaction of Chitosan with TEOF

90 To 100 mL of toluene containing chitosan film (4x5 cm), TEOF (0.01mol), was added. The
91 reaction mixture was refluxed for 12hr. The obtained films were refluxed in ethanol for 1h.

92 2.3.2. Preparation of CS-CA and CS-CN: Reaction of chitosan with chloroacetone and 93 chloroacetonitrile

94 To 100 mL of toluene containing film of chitosan (4x5 cm) and sodium carbonate (2g),
95 chloroacetone or chloroacetonitrile (0.01 mol) was added (**Why mole ratio fixed as**
96 **0.01 mol??? Need an explanation**). The reaction mixture was reflux for 10 hr. The
97 obtained modified chitosan derivative films were refluxed in ethanol for 1hr.

98 2.3.3. Preparation of CSTEOP-ATP, CSTEOP-AP and CSTEOP-PH: Reaction of CS-TEOF 99 with amines

To 100 mL of toluene/ethanol mixture (3:1) containing film of CS-TEOF (4x5 cm), 2-aminothiophenol or 2-aminophenol, phenyl hydrazine (0.01 mol) was added (Why mole ratio fixed as 0.01 mol??? Need an explanation), and then heated under reflux for 10hr. The produced modified chitosan derivative films were refluxed in ethanol for 1hr.

2.3.4. Preparation of metallo-polymer complex

To hot solution of metal nitrate salts (0.01 mole) (Why mole ratio fixed as 0.01 mol??? Need an explanation) in DMF, CS-CA film (4x5 cm) was submerged and then heated under reflux for 5hr. The metallo-polymer complex films obtained were purified by heat in ethanol for 1hr.

2.3.5. Ion selectivity

To an aqueous solution containing (0.01 mol) of metal nitrate mixture ($M = Cu^{2+}, Cd^{2+}, Fe^{3+}, Co^{2+}, Pb^{2+}, Mn^{2+}, Hg^{2+}, Cr^{3+}, Pd^{2+}$), modified chitosan film was added (Why mole ratio fixed as 0.01 mol??? Need an explanation). The mixture was left for 24hr with stirring. The reaction product was filtered, and ICP measurements of the metal contents for the original solution and the obtained filtrate were measured.

2.3.6. Concentration of metal ions in the Gulf Water Samples

5L of real sample of Gulf water was pre-concentrated to a final volume of 150 mL. The metal ions concentration was determined by ICP-OES. 150 mL of the pre-concentrated sample was divided into three portions of 50.0 mL and then treated with modified chitosan film under a shaking rate of 350 rpm for 24hr. The mixture was filtered and the polymer film washed several times with doubly distilled water. The results of ICP-OES measurements before and after treatment and the percent of metal uptake onto the chelating polymer are computed according to the following equation;

$$\% \text{ sorption} = [C_i - C_f / C_i] \times 100 \quad \text{eq. (1)}$$

Where, C_i and C_f are the initial and final concentration of the metal ion (mg/L or in $\mu\text{g/L}$) before and after treatment, respectively. The results are the average of triplicate measurements, and the precision in most cases is very close to $\pm 1.5 \%$.

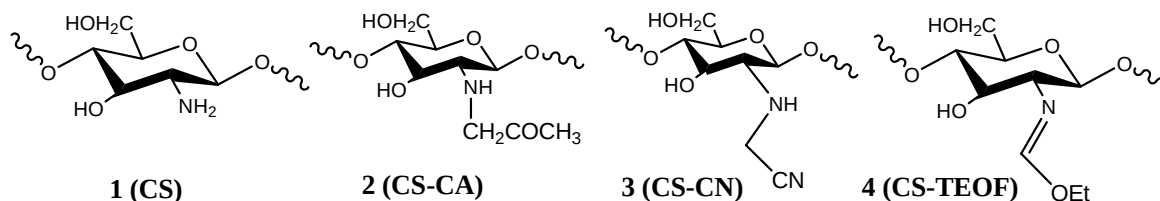
2.3.7. Procedure of antimicrobial tests

A solution or suspension of the tested modified polymers and polymer metal complexes (0.01 mole), [prepared by dissolving 400 µg/mL (w/v) in sterile DMSO] (Why mole ratio fixed as 0.01 mol??? Need an explanation) was poured aseptically in a well of six mm diameter made by a borer in the seeded agar medium. After transferring *via* pipette the same volume in wells of all tested microorganisms, bacteria test plates were incubated at 37°C for 24 hr and fungal test plates were incubated at 25°C for 48 hr. The activities were expressed as inhibition zones (mm, diameter, as clear areas). The least concentration, which showed inhibitory effect on any specific microorganism, was considered as the minimum inhibitory concentration (MIC) which was determined using streptomycin and mycostatin (50 µg/mL) as the references.

3. RESULTS AND DISCUSSION

3.1. Modification of Chitosan and Characterization

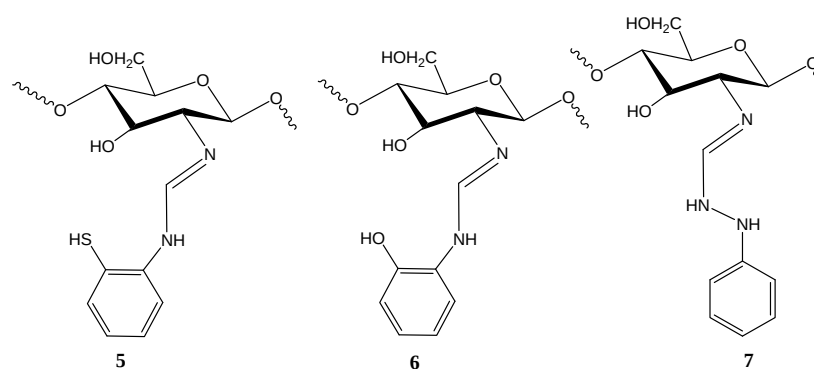
Chitosan (**1**) alkylated with chloroacetone and chloroacetonitrile in the presence of sodium carbonate to give the *N*-alkylated products **2** and **3**, respectively (Scheme 2). The reaction product was established based on the IR spectrum for example modified polymer, CS-CA (**2**) displayed two carbonyl bands at 1678 and 1649 cm⁻¹, while CS-CN (**3**) showed the cyano and amide carbonyl groups at 2244 and 1649 cm⁻¹, respectively (Table 1). Moreover, chitosan reacted readily with triethyl orthoformate to give, CS-TEOF, **4**. The structure of derivative **4** was established based on IR which reveals the presence of new band at 1659 cm⁻¹ for C=N, in addition to the presence of new band at 3098 for imine-H and NH band at 3343 cm⁻¹ for amide NH₂ and disappearance of amino group of chitosan which exhibited two bands at 3393, 3168 cm⁻¹ (Table 1).



Scheme 2. Modification of chitosan with different organic reagents

CS-TEOF reacted with 2-aminothiophenol, 2-aminophenol and phenylhydrazine in a mixture of toluene/ethanol (1:1) to afford the modified chitosan **5-7** (scheme 3). The structure of

compounds CSTEOP-ATP (5) was assigned as reaction product depending on the elemental analysis which showed a sulfur analysis 6.72%. It is worth to be mentioning that the sulfur percent increases with the duration of heating. Moreover, the IR reveals the presence of new aromatic C-H band at 3099. Derivative 6 was established based on IR which showed the hydroxyl and NH groups at 3459, 3406, 3335-3210 cm^{-1} in addition to new band at 3135 cm^{-1} which assigned for the aromatic C-H. Similarly, compound 7 was established as a reaction product (what was % of functionalization???, at least roughly or anticipated % of functionalisation) (any studies carried out on these information may be included).



Scheme 3. Synthesis of N-N, N-O and N-N chelating centers

3.2. Complex Formation

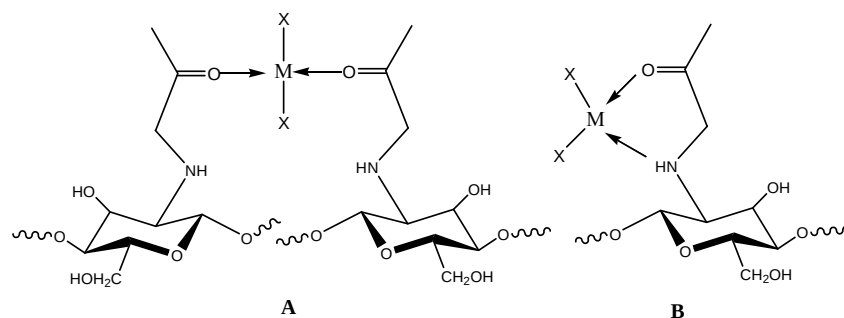
The modified chitosan CS-CA was refluxed with metal salt solution in suitable solvent to give the corresponding complexes. The structures of the obtained complexes were established based elemental analysis, FT-IR (*cf.* Table 1) and XPS data. The XPS of metallo-polymer complex structure confirm the presence of the metal chelation and it refers to the center of chelation through study the binding energy.

3.3. X-Ray Photoelectrons Spectroscopy (XPS)

Modified chitosan CS-CA was heated with different metal ions e. g. Cd(II), Cu(II) and Pd(II) to give the corresponding complexes. XPS Fig.(1A) shows the core level spectra of Cd (3d) consisting of the Cd3d_{5/2} 'Cd3d_{5/3} components located at 405.45 and at 412.08 eV respectively. The structure A or B was suggested as a reaction product. The results suggest the binding energy of Cd is attributed to Cd²⁺ bonding state [35] and assigned to Cd-O bond.

Thus, structure B was ruled out while, structure A (scheme 4) was assigned as a reaction product.

The peak at 934.07 eV in the Cu2p XPS spectrum Fig. (1B) suggested the coordination bonding between Cu and the nitrogen of polymer (Cu-N). The Cu2p XPS spectrum evidenced Cu⁺ (932.7eV) and Cu²⁺ (934.07eV) in polymer-Cu complex, which confirmed that metals may be simultaneously redox-transformed when complexed with polymer. The peak at binding energy 953.8 eV assigned for Cu-O bond. Structure B (scheme 4) was assigned as a reaction product. Fig. (1C) shows the XPS spectra of PdCl₂ with CSCA-PdCl₂. The XPS data shows that the Pd3d centered at 337.1 and 342.5 eV were assigned to Pd(II) species and mostly for Pd-O bond. Structure A (scheme 4) was assigned as a reaction product. Figure 2 shows the XPS spectra of Pd(II) with CSCA-PdSO₄ complex. Similarly, Structure A was assigned for the CSCA-PdSO₄ complex. In addition, the elemental analysis of CSCA-PdSO₄ complex showed 5.97 sulfur %. All binding energy values for metal-polymer complex are slightly different than what was mentioned in literature. The difference could be attributed to the coordination action of metal and modified chitosan polymer. The Cu(II) complex (structure B) different than that of Cd(II) and Pd(II) (structure A) may be due to the difference of ionic radius in addition, the ionic radius of Cu(II) more fit with the bidentate chelating center N-O.



Scheme 4. Suggested complex structure depending on XPS data

3.4. Ion Selectivity

A mixture of different metal salts was treated with different polymers and ICP measurement for the blank solution and that of the solution after treatment for 24hr with polymer was measured. The differences refer to the metal uptake value.

The results of ICP-OES measurements before and after treatment and the percent of metal uptake onto the chelating polymer are reported in Tables 2-6 and computed according to equation (1).

Atomic absorption measurements for a mixture of the modified polymers CSTEOPH with a mixture of metal nitrate, $M = \text{Ca}^{2+}, \text{Cd}^{2+}, \text{Co}^{2+}, \text{Cr}^{3+}, \text{Cu}^{2+}, \text{Fe}^{3+}, \text{Mg}^{2+}, \text{Mn}^{2+}, \text{Ni}^{2+}$ or Pb^{2+} before and after treating with the polymer are given in Tables 2. The polymer selectivity order of modified polymer CSTEOPH could be arranged as follows: $\text{Pb} > \text{Co} > \text{Ca} > \text{Cd} > \text{Mn} > \text{Fe} > \text{Cu} > \text{Cr} > \text{Mg}$

It was clear from the data that CSTEOPH was selected for six ions (Pb, Co, Ca, Cd, Mn and Fe) and less selective to Cu, Cr and Mg. The study of metal uptake of Hg(II) from aqueous solution by CSTEOPH is reported in Table 3. The sorption of Hg was decreased with increasing the duration to 48h instead of 24h and also decreases upon heating for two hours. Furthermore, the polymer selectivity order of modified polymer CSTEOPAP was reported in Table 4 could be arranged as follows: $\text{Pb} > \text{Co} > \text{Fe} > \text{Mn} > \text{Cd} > \text{Ca} > \text{Cu} > \text{Cr} > \text{Mg}$. The data reflected that CSTEOPAP was selected for Pb, Co, Fe, Mn, Cd and Ca and less selective to Cu, Cr and Mg. The ICP data of modified chitosan CSTEOPATP was reported in Table 5. The CSTEOPATP selectivity could be arranged as follows: $\text{Pb} > \text{Co} > \text{Ca} > \text{Fe} > \text{Mn} > \text{Cd} > \text{Cr} > \text{Mg} > \text{Cu}$. The data showed that CSTEOPATP was selected for Pb, Co, Ca, Fe, Mn and Cd, The CSTEOPATP less selective for Cr, Mg and Cu. Generally, the data showed that the modified chitosan was selective for some metal ions depending on the type of chelating center furnished from the modification process e.g. N-N, N-O, O-O and N-S. The metal ions Fe, Co and Cu preferred N-O while Pb, Cr, Ca, Ni, Cd and Pd preferred O-O chelating center. On the other hand, copper ions more preferred N-S chelating center than N-N in the modified chitosan.

3.5. Determination of Metal Ions in Gulf Water Samples

Using of the modified polymer for the Gulf Ocean water analysis showed that the CS-CA was able to uptake many metal ions (such as Na, K, Mg, Ca, Co, Ni, Cu, Cd, Pb, Fe and Hg) from the Gulf ocean water. The ions Cu, Ni, Fe, Hg, and Co was measured in $\mu\text{g/L}$, while Na, K, Mg and Ca was measured in mg/L . The results of ICP-OES measurements before and after treatment and the sorption percent onto the chelating polymer are reported in Table 6 and calculated according to equation (1)

3.6. The Structures and Morphology

Generally, the change in the surface morphology may be due to modification of the polymer or/and the introduction of the metal ions in the polymer matrix. SEM was studied to confirm the chelation of the metal ions with the polymeric chain and to study how the change of surface occurred. The SEM of modified derivatives CS-CA and there metallo-polymer complexes with different metal ions are shown in Fig.3, with 10000 magnifications. It is observed that the crystallinity of the metal complexes of CSCA-Cu complex, CSCA-Cd complex and CSCA-Pd complex is more than that of CS and its modified derivatives CS-CA. The SEM for both CS and CS-CA are showed a smooth surface. This means that the chelation mostly occurred on the surface of the polymer. Fig. 3A SEM micrograph of CS-CA seemed to be smoothed surface somewhat similar to that of chitosan. Figure 3B and 3C SEM micrograph of CSCA-Cu complex and CSCA-Cd complex. The microgram of the CSCA-Cu complex and CSCA-Cd complex are similar in surface which looks like tubes in its shape. On the other hand, Fig. 3D SEM micrograph of CSCA-Pd complex. Pd-complex showed a different change in the surface as seen from the figure as compared by Cu-complex and Cd-complex.

3.7. Biological Activity

The well known diverse biological activities of compounds containing sulfur and nitrogen as components of organic compounds [36-40], in addition to chitosan prompted us to test and study the antibacterial activities and antifungus of some of the modified chitosan and metallopolymer complex. Table 7 shows that most of the tested compounds had high to low activity against tested microorganisms. It was reported that chitosan derivatives killed bacteria through disrupting the inner and the outer membrane [41].

From Table (7) most of the tested compound showed high to low effect against bacteria and fungi. Thus, CS-CN, CSTEOP-AP and CSTEOP-ATP exhibit severe effect against *S. aureus*, while CS-CN and CSTEOP-ATP showed severe effect against *B. subtilis*. In addition, CS-CN, CS-TEOFF, CSTEOP-ATP and CSTEOP-ATP exhibit severe effect against *E. coli*. The tested compound showed medium to low effect against *Salmonella*, while CS-TEOF, CSTEOP-PH and CSTEOP-ATP showed high effect against *Candida albicans*. CSCA-Cu complex showed a severe effect against *S. aureus*, *E. coli* and *Salmonella*. Investigations of CS-CA showed a moderate to slight effect in case of bacteria and non effect was reported against both tested fungi.

4. CONCLUSION

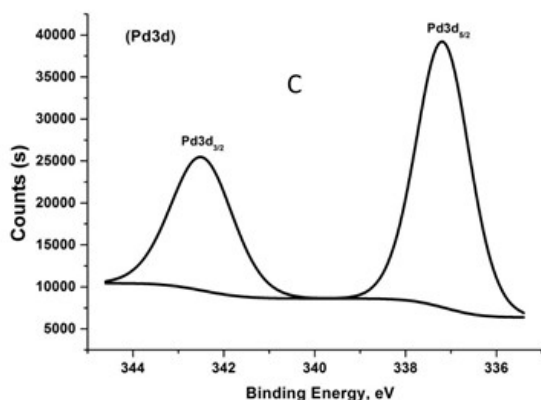
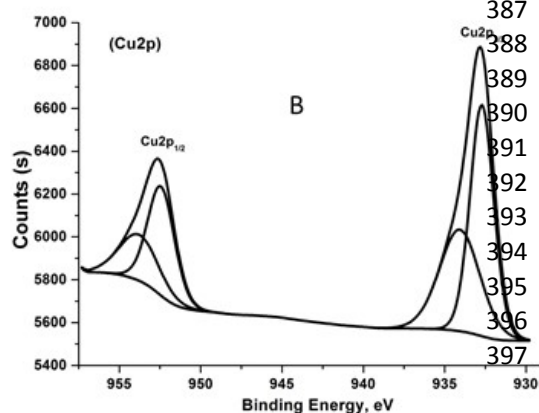
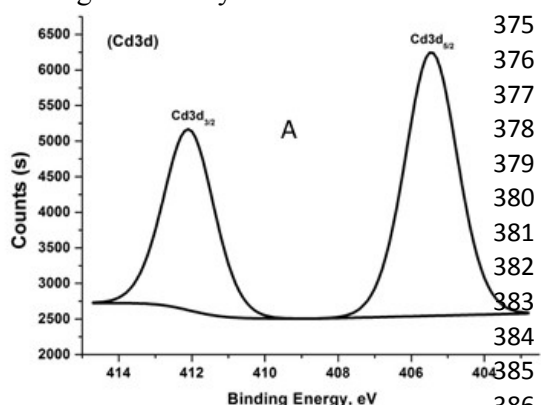
In this article chitosan was updated through modification under normal condition. The modifications carried out by the reaction of different organic reagents with amino group as active site in chitosan. Further reactions with the modified chitosan were also successes and leading to produce different chelating active centers and biologically active derivatives. The using of the modified chitosan in waste treatment was investigated. The data showed that the modified chitosan was selective for some metal ions depending on the type of chelating center furnished from the modification process e.g. N-N, N-O, O-O and N-S. The metal ions Fe, Co and Cu preferred N-O while Pb, Cr, Ca, Ni, Cd and Pd preferred O-O chelating center. Copper ion preferred N-S chelating center than N-N in the modified chitosan. The removal capacity by modified chitosan ligands for Ca^{2+} , Cd^{2+} , Fe^{3+} , Co^{2+} , Pb^{2+} , Mn^{2+} exceed 80%. The modifications and complexation have a significant impact on the morphology of the produced products. Some of the modified chitosan and their complexes have showed a significant effect on fungi, gram positive and gram negative bacteria.

REFERENCES

1. Alexander P, Svetlana B Chitosan and Its Derivatives as Highly Efficient Polymer Ligands. *Molecules* 2016;21: 330-335
2. Pestov A, Bratskaya S Chitosan and Its Derivatives as Highly Efficient Polymer Ligands, *Molecules* 2016;21:330-365 and references cited therein.
3. Cheung RCF, Ng TB, Wong JH, Chan WY Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications, *Mar. Drugs* 2015;13:5156-5186
4. Li B, Fang Y, Shan CL, Ibrahim M, Xie GL, Wang YL, Sun GC Differential Effect of Metal Ions on Antibacterial Activity of Chitosan Against *Burkholderia cenocepacia*. *Asian J. Chem.* 2013;25: 891-894.
5. Hadi AG Adsorption of Cd(II) ions by synthesize chitosan from fish shells, *British J. Sci.* 2012;5:33-38.
6. Mekahlia S, Bouzid B Chitosan-Copper (II) complex as antibacterial agent: synthesis, characterization and coordinating bond- activity correlation study, *Physics procedia* (Proceedings of the JMSM 2008 Conference), 2009;2:1045-1053.
7. Yin X, Zhang X, Lin Q, Feng Y, Yu W, Zhang Q Metal-coordinating controlled oxidative degradation of chitosan and antioxidant activity of chitosan-metal complex. *ARKIVOC* 2004;ix:66-78.
8. Prashanth KVV, Tharanathan RN Chitin/chitosan: modifications and their unlimited application potential—an overview, *Trends in Food Science & Technology* 2007;1: 117-131.
9. Badawy MEI, El-aswad AFI Insecticidal Activity of Chitosans of Different Molecular Weights and Chitosan-metal Complexes against Cotton Leaf worm *Spodoptera littoralis* and Oleander Aphid *Aphis nerii*, *Plant Protect. Sci.* 2012;48:131-141.
10. Shigemasa Y, Minami S Applications of chitin and chitosan for biomaterials, *Biotechn. Genet. Eng. Rev.* 1996;13:383-420.

11. Wang X, Du Y, Liu H Preparation, characterization and antimicrobial activity of chitosan-Zn complex, *Carbohydr. Polym.* 2004;56:21-26.
12. Higazy A, Hashem M, Elshafei A, Shaker N, Abdel Hady M Development of antimicrobial jute packaging using chitosan and chitosan-metal complex, *Carbohydr. Polym.*, 2010;79:867-874.
13. Wang X, Du Y, Fan L, Liu H, Hu Y Chitosan metal complexes as antimicrobial agent: Synthesis, characterization and structure-activity study. *Polym. Bull.* 55:105-113.
14. Sy Q, Daj W, Forster Cf (1998) The use of sago waste for the sorption of lead and copper, *Water SA* 2005;24:251-256.
15. Ahmad A, Ghufuran R, Faizal WM Cd(II), Pb(II) and Zn(II) Removal from Contaminated Water by Biosorption Using Activated Sludge Biomass. *CLEAN – Soil, Air. Water* 2010;38:153-158.
16. Zhang L, Zeng Y, Cheng Z Removal of heavy metal ions using chitosan and modified chitosan: A review. *J. Molecular Liq.* 2016;214:175-191.
17. Guibal E Interactions of metal ions with chitosan-based sorbents: A review. *Sep. Purif. Technol.* 2004;38:43–74.
18. Varma AJ, Deshpande SV, Kennedy JF Metal complexation by chitosan and its derivatives: A review. *Carbohydr. Polym.* 2004;55:77–93.
19. Guibal E, Vincent T, Navarro R Metal ion biosorption on chitosan for the synthesis of advanced materials. *J. Mater. Sci.* 2014;49:5505–5518.
20. Kurita K Controlled functionalization of the polysaccharide chitin. *Prog. Polym. Sci.* 2001;26:1921–1971.
21. Kurita K Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar. Biotechnol.* 2006;8:203–226.
22. Wang J, Chen C Chitosan-based biosorbents: Modification and application for biosorption of heavy metals and radionuclides. *Bioresour. Technol.* 2014;160:129–141.
23. Boamah PO, Huang Y, Hua M, Zhang Q, Wu J, Onumah J, Sam-Amoah LK, Boamah PO Sorption of heavy metal ions onto carboxylate chitosan derivatives—A mini-review. *Ecotoxicol. Environ. Saf.* 2015;116:113–120.
24. Fu F, Wang Q Removal of heavy metal ions from wastewaters: A review. *J. Environ. Manag.* 2011;92:407- 418.
25. Ali R K, Bahare R, Azam K Effective removal of a cobalt-tetrasulfonated phthalocyanine dye from an aqueous solution with a novel modified chitosan-based superabsorbent hydrogel. *J. Appl. Polym. Sci.* 2018, 135, 46167.
26. Shariful MI, Sepehr T, Mehrali M, Ang BC, Amalina MA Adsorption capability of heavy metals by chitosan/poly(ethylene oxide)/activated carbon electrospun nanofibrous membrane. *J. Appl. Polym. Sci.* 2018;135(7):45851. DOI: 10.1002/app.45851
27. Iyengar U, Avs PR Hexavalent chromium interaction with chitosan, *J. Appl. Polym. Sci.* 1990;39:739-747.
28. Findon A, McKay G, Hs. Blair Hs Transport studies for the sorption of copper ions by chitosan. *J. of Environ. Sci. and Health* 1993;A28:173-185.
29. Schmuhl R, Krieg HM, Keizer K Adsorption of Cu(II) and Cr(VI) ions by chitosan: Kinetics and equilibrium studies, *Water SA* 2001;27:1-7.
30. Kyoon H, Park NY, Lee SH, Meyers SP Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Inter. J. Food Microbiology* 2002;74:65-72

31. Asano T Proc. First Int. Conf. Chitin/Chitosan, R. A. A Muzarelli and E. R. Pariser, eds, Cambridge, MA: MIT Sea Grant report, 1978;78-7:231-252.
32. Allan GG, Altman LC, Bensinger RE, Ghosh DK, Hirabayashi Y, Neogi AN, Neogi S In Chitin, Chitosan and Related Enzymes, J. P. Zikakis, ed., Academic Press, Inc., 1984;119 - 133.
33. Chen CS, Liao WY, Tsa GJ Antibacterial Effects of N-Sulfonated and N-Sulfobenzoyl Chitosan and Application to Oyster Preservation. J. Food Prot. 1998;61:1124-1128.
34. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J. Biomed. Mater. Res. 2000;52:662-668.
35. Thovhogi N, Park E, Manikandan E, Maaza M, Gurib-Fakim A. Physical properties of CdO nanoparticles synthesized by green chemistry via *Hibiscus Sabdariffa* flower extract. J. Alloys and Compd. 2016;655:314-320.
36. Keri RS, Patil MR, Patil SA, Budagumpi S Comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry Eur. J. Med. Chem. 2015;89:207-251.
37. Hisamoddin SZK, Sanchaity P, Sharma PY, Patel NU Benzothiazole the molecule of diverse biological activity. Pharma Sci. Monitor 2014;5:207-225.



38. Yang Y, Cui M. Radiolabeled bioactive benzoheterocycles for imaging β -amyloid plaques in Alzheimer's disease. Eur. J. Med. Chem. 2014;87:703-721.
39. Manoj NB, Mayuri AB, Hitesh DP Synthetic Strategies for Fused Benzothiazoles: Past, Present, Future, Synth. Comm. 2014;44:2427-2457.
- W, Xu P, Wang W, Liu Q preparation and antibacterial activity of a water-soluble chitosan derivatives, Carbohydr. Polym. 2002;50:35-40
- J-Y, Kim S-K Chitosan derivatives killed bacteria by disrupting the outer and inner membrane, J. Agric. Food Chem. 2006;54:6629-6633.

402

403

404

405

406

407

408

409

410

411

412

413

414

415

416

417

418 **Fig. 1.** Xps data of modified CS complex (A) CSCA-Cd complex; (B) CSCA-Cu complex
419 and (C) CSCA-Pb complex

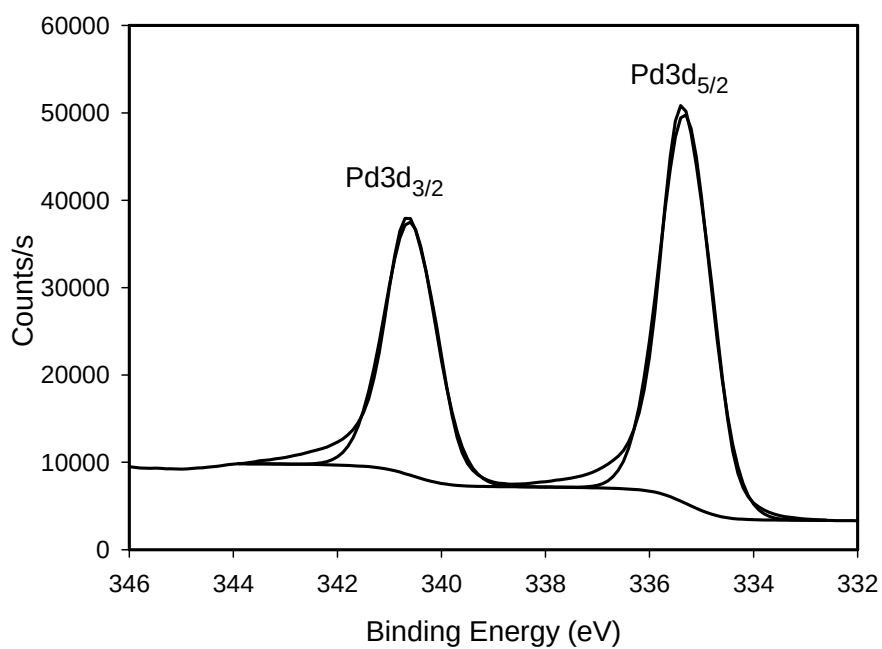


Fig. 2. XPS data of CSCA-PdSO₄ complex

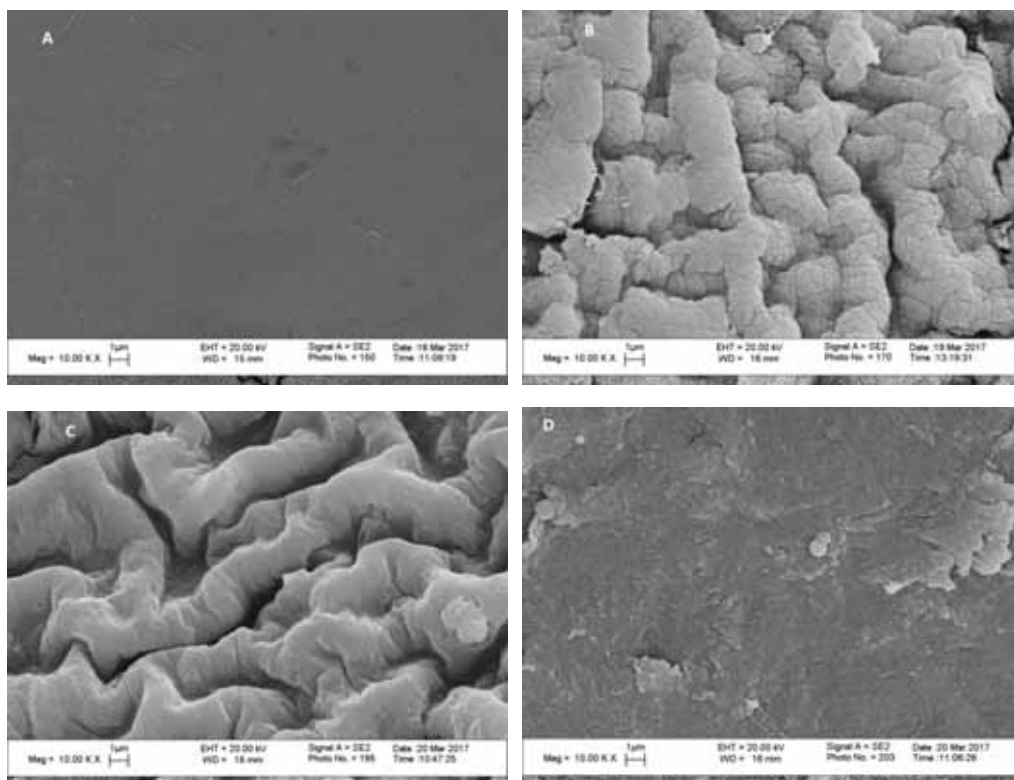


Fig. 3. SEM image with 10000 magnification (A) CS-CA; (B) CSCA-Cu complex; (C) CSCA-Cd complex; (D) CSCA-Pd complex

427 **Table 1** Elemental analysis and IR of chitosan, modified chitosan and metal complexes

Compound	C%	H%	N%	S%	IR (cm ⁻¹)
CS	39.04	7.53	5.88		3393, 3168 (NH, NH ₂); 2924, 2880 (CH), 1642 (C=O), 1554, 1408, 1321
CS-CA	41.12	7.02	5.62		3348, 3294, 3235, 3199, 2881, 1678, 1649, 1559, 1380, 1317, 1257, 1150, 1104, 1009, 896, 614
CS-CN	39.07	7.39	6.76		3358, 3216, 2886, 2244, 1649, 1551, 1377, 1316, 1150, 1112, 1025, 896, 612
CSTEOF-PH	48.74	6.90	7.10		3343, 3098, 2993, 2882, 1652, 1647, 1552, 1384
CSTEOF -ATP	55.25	7.22	9.51	6.72	3459, 3342, 3285, 3200, 3135, 2884, 1650, 1561, 1381
CSTEOF -AP	41.80	6.52	6.97		3469, 3406, 3335, 3283, 3248, 3210, 3099, 2926, 2884, 1705, 1653, 1559, 1406, 1324
CSTEOF S-AP-Cu	39.21	6.36	7.99		3469, 3406, 3335, 3283, 3248, 3210, 3099, 2926, 2884, 1653, 1559, 1406, 1324
CSCA-PdSO ₄	33.53	6.43	5.32	5.97	3596, 2648, 1630, 1590, 1513, 1421, 1350
CSCA-Cu	33.56	6.10	8.72		3426, 3345, 3254, 3150, 3107, 3008, 2977, 2838, 2080, 1680, 1632, 1535
CSCA-Cd	32.17	5.80	8.54		3636, 2680, 2076, 1727, 1671, 900
CSCA-PdCl ₂	39.79	6.75	6.09		3624, 2487, 2129, 1686

428

429 **Table 2** Ion selectivity (ICP) of different metal ions from aqueous solution by CSTEOF-PH

Metal ion	(C _i) mg/L	(C _f) mg/L	(C _i - C _f)	Sorption %
Ca	1642.5	184.5	1458	88.8
Cd	2366.8	336.8	2030	85.8
Co	1836	161.3	1674.7	91.2
Cr	2382.6	912.5	1470.1	61.7
Cu	1877.5	703.8	1173.7	62.5
Fe	2420.56	461.7	1958.86	80.9
Mg	1485.5	686.1	799.4	53.8
Mn	1795.5	265.1	1530.4	85.2
Pb	3315.1	281.7	3033.4	91.5

430

(mention as ions in the table)

431 **Table 3** Ion selectivity of Hg(II) ions from aqueous solution by CSTEOF-PH

Reaction	C _i	C _f	C _i -C _f	Sorption
----------	----------------	----------------	--------------------------------	----------

time (h)	mg/L	mg/L	(units???)	%
After 24h	165398	24236	141162	85.3
After 48h	165398	87873	77525	46.9
After heating 2h	165398	99123	66275	40.1

432

433 **Table 4** Ion selectivity (ICP) of different metal ions from aqueous solution by CSTEOf-AP

Metal ion	C _i mg/L	C _f mg/L	C _i - C _f Unit???	Sorption %
Ca	1642.5	248.6	1393.9	84.9
Cd	2366.8	351.2	2015.6	85.2
Co	1836	222.6	1613.4	87.9
Cr	2382.6	876.5	1506.1	63.2
Cu	1877.5	612.5	1265	67.4
Fe	2420.56	326.1	2094.46	86.5
Mg	1485.5	650.3	835.2	56.2
Mn	1795.5	244.9	1550.6	86.4
Pb	3315.1	349.5	2965.6	89.5

434

(mention as ions in the table)

435 **Table 5** Ion selectivity of different metal ions from aqueous solution by CSTEOf-ATP

Metal ions	C _i mg/L	C _f mg/L	C _i - C _f Unit???	Sorption %
Ca	1642.5	199.2	1443.3	87.9
Cd	2366.8	463.8	1903	80.4
Co	1836	215.7	1620.3	88.3
Cr	2382.6	789.5	1593.1	66.9
Cu	1877.5	555.8	1321.7	70.4
Fe	2420.56	358.4	2062.16	85.2
Mg	1485.5	487.3	998.2	67.2
Mn	1795.5	296.7	1498.8	83.5
Pb	3315.1	358.8	2956.3	89.2

436

(mention as ions in the table)

437 **Table 6** ICP-OES Results of a Gulf Ocean Water Sample before and after Treatment with
438 the Chelating polymer CS-CA

439

Metal ion	C_i units???	C_f units???	$C_i - C_f$ units???	% of metal uptake
${}^a\text{Na}^+$	16427.55	2451.6	13975.95	85.1
${}^a\text{K}^+$	763.9	164.9	599	78.4
${}^a\text{Mg}^{2+}$	1611.4	123.3	1488.1	92.3
${}^a\text{Ca}^{2+}$	509.5	31.3	478.2	93.9
${}^b\text{Co}^{2+}$	2.54	0.156	2.384	93.9
${}^b\text{Ni}^{2+}$	13.75	0.177	13.573	98.7
${}^b\text{Cu}^{2+}$	13.7	2.3	11.4	83.2
${}^b\text{Cd}^{2+}$	BDL	ND		
${}^b\text{Pb}^{2+}$	74.2	15.6	58.6	79.0
${}^b\text{Fe}^{3+}$	1.07	0.11	0.96	89.7
${}^b\text{Hg}^{2+}$	15.1	1.2	13.9	92.1

440 *a*: C_i in mg/L; *b*: C_i in $\mu\text{g/L}$; ND: not detected; BDL: below detection limit.

441

442 **Table 7** *In vitro* bactericidal and fungicidal activity of some newly synthesized compounds

Compound	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Candida albicans</i>
CS-CA	++	++	+	Nil	Nil
CS-CN	+++	+++	+++	++	++
CS-TEOF	++	++	+++	+	+++
CS-TEOF-PH	++	++	++	++	+++
CS-TEOF-ATP	+++	+++	+++	++	+++
CS-TEOF-AP	+++	++	+++	++	+
CSCA-Cu complex	+++	++	+++	+++	++

443 Slight effect = +; Moderate effect = ++; Severe effect = +++p??? (Explain in the

444 text)